group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof. Also, the PS128-specific polynucleotide may be attached to a solid phase prior to performing the method.

The present invention also provides a method for detecting PS128 mRNA in a test sample, which comprises performing reverse transcription (RT) with at least one primer in order to produce cDNA, amplifying the cDNA so obtained using PS128 oligonucleotides as sense and antisense primers to obtain PS128 amplicon, and detecting the presence of the PS128 amplicon as an indication of the presence of PS128 mRNA in the test sample, wherein the PS128 oligonucleotides have at least 50% identity with a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof. Amplification can be performed by the polymerase chain reaction. Also, the test sample can be reacted with a solid phase prior to performing the method, prior to amplification or prior to detection. This reaction can be a direct or an indirect reaction. Further, the detection step can comprise utilizing a detectable label capable of generating a measurable signal. The detectable label can be attached to a solid phase.

E1 Cont

The present invention further provides a method of detecting a target PS128 polynucleotide in a test sample suspected of containing target PS128 polynucleotides, which comprises (a) contacting the test sample with at least one PS128 oligonucleotide as a sense primer and at least one PS128 oligonucleotide as an anti-sense primer, and amplifying same to obtain a first stage reaction product; (b) contacting the first stage reaction product with at least one other PS128 oligonucleotide to obtain a second stage reaction product, with the proviso that the other PS128 oligonucleotide is located 3' to the PS128 oligonucleotides utilized in step (a) and is complementary to the first stage reaction product; and (c) detecting the second stage reaction product as an indication of the presence of a target PS128 polynucleotide in the test sample. The PS128 oligonucleotides selected as reagents in the method have at least

50% identity with a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof. Amplification may be performed by the polymerase chain reaction. The test sample can be reacted either directly or indirectly with a solid phase prior to performing the method, or prior to amplification, or prior to detection. The detection step also comprises utilizing a detectable label capable of generating a measurable signal; further, the detectable label can be attached to a solid phase. Test kits useful for detecting target PS128 polynucleotides in a test sample are also provided which comprise a container containing at least one PS128 specific polynucleotide selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof. These test kits further comprise containers with tools useful for collecting test samples (such as, for example, blood, urine, saliva and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials, such as papers, cloths, swabs, cups, and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens.

E' cont.

The present invention also provides a purified polynucleotide or fragment thereof derived from a PS128 gene. The purified polynucleotide is capable of selectively hybridizing to the nucleic acid of the PS128 gene, or a complement thereof. The polynucleotide has at least 50% identity with a sequence selected from the group consisting of (a) SEQUENCE ID NO: 1, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and complements thereof, and (b) fragments of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, and SEQUENCE ID NO: 3. Further, the purified polynucleotide can be produced by recombinant and/or synthetic techniques. The purified recombinant polynucleotide can be

contained within a recombinant vector. The invention further comprises a host cell transfected with the recombinant vector.

The present invention further provides a recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from PS128. The nucleic acid sequence has at least 50% identity with a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof. The nucleic acid sequence is operably linked to control sequence compatible with a desired host. Also provided is a cell transfected with this recombinant expression system.

The present invention also provides a polypeptide encoded by PS128. The polypeptide can be produced by recombinant technology, provided in purified form, or produced by synthetic techniques. The polypeptide comprises an amino acid sequence which has at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof.

E1 cont

Also provided is an antibody which specifically binds to at least one PS128 epitope. The antibody can be a polyclonal or monoclonal antibody. The epitope is derived from an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. Assay kits for determining the presence of PS128 antigen or anti-PS128 antibody in a test sample are also included. In one embodiment, the assay kits comprise a container containing at least one PS128 polypeptide having at least 50% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. Further, the test kit can comprise a container with tools useful for collecting test samples (such as blood, urine, saliva, and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing urine or stool samples. Collection materials such as papers, cloths, swabs, cups, and the like, may optionally be treated to avoid

denaturation or irreversible adsorption of the sample. These collection materials also may be treated with or
contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Also, the polypeptide can be attached to a solid phase.

Another assay kit for determining the presence of PS128 antigen or anti-PS128 antibody in a test sample comprises a container containing an antibody which specifically binds to a PS128 antigen, wherein the PS128 antigen comprises at least one PS128-encoded epitope. The PS128 antigen has at least about 60% sequence similarity to a sequence of a PS128-encoded antigen selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. These test kits can further comprise containers with tools useful for collecting test samples (such as blood, urine, saliva, and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. These collection materials also may be treated with, or contain, preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. The antibody can be attached to a solid phase.

A method for producing a polypeptide which contains at least one epitope of PS128 is provided, which method comprises incubating host cells transfected with an expression vector. This vector comprises a polynucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 50% identity with a PS128 amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof.

A method for detecting PS128 antigen in a test sample suspected of containing PS128 antigen also is provided. The method comprises contacting the test sample with an antibody or fragment thereof which specifically binds to

at least one epitope of PS128 antigen, for a time and under conditions sufficient for the formation of antibody/antigen complexes; and detecting the presence of such complexes containing the antibody as an indication of the presence of PS128 antigen in the test sample. The antibody can be attached to a solid phase and may be either a monoclonal or polyclonal antibody. Furthermore, the antibody specifically binds to at least one PS128 antigen selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof."

E1

Please amend page 8, line 1 through page 9, line 12 to read as follows:

"Another method is provided which detects antibodies which specifically bind to PS128 antigen in a test sample suspected of containing these antibodies. The method comprises contacting the test sample with a polypeptide which contains at least one PS128 epitope, wherein the PS128 epitope comprises an amino acid sequence having at least 50% identity with an amino acid sequence encoded by a PS128 polynucleotide, or a fragment thereof. Contacting is carried out for a time and under conditions sufficient to allow antigen/antibody complexes to form. The method further entails detecting complexes which contain the polypeptide. The polypeptide can be attached to a solid phase. Further, the polypeptide can be a recombinant protein or a synthetic peptide having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof.

E2

The present invention provides a cell transfected with a PS128 nucleic acid sequence that encodes at least one epitope of a PS128 antigen, or fragment thereof. The nucleic acid sequence is selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof.

A method for producing antibodies to PS128 antigen also is provided, which method comprises administering to an individual an isolated immunogenic polypeptide or fragment thereof. The isolated immunogenic polypeptide comprises at least one PS128 epitope, and is administered in an amount sufficient to produce an immune response. The isolated immunogenic polypeptide comprises an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof.

Another method for producing antibodies which specifically bind to PS128 antigen is disclosed, which method comprises administering to an individual a plasmid comprising a nucleic acid sequence which encodes at least one PS128 epitope derived from an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. The plasmid is administered in an amount such that the plasmid is taken up by cells in the individual and expressed at levels sufficient to produce an immune response.

Also provided is a composition of matter that comprises a PS128 polynucleotide of at least about 10-12 nucleotides having at least 50% identity with a sequence selected from the group consisting of (a) SEQUENCE ID NO: 1, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and complements thereof, and (b) fragments of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, and SEQUENCE ID NO: 3. The PS128 polynucleotide encodes an amino acid sequence having at least one PS128 epitope. Another composition of matter provided by the present invention comprises a polypeptide with at least one PS128 epitope of about 8-10 amino acids. The polypeptide comprises an amino acid sequence having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. Also provided is a gene, or fragment thereof, coding for a PS128 polypeptide which has at least 50% identity to SEQUENCE ID NO: 12; and a gene, or a fragment thereof,

E2 comprising DNA having at least 50% identity with SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5."

Please amend page 9 lines 15-22 to read as follows:

"Figure 1 shows the nucleotide alignment of clones 824664 (SEQUENCE ID NO: 1), g2356393 (SEQUENCE ID NO: 2), 1792994 (SEQUENCE ID NO: 3), the full-length sequence of clone 824664 (designated as clone 824664IH (SEQUENCE ID NO: 4)), and the consensus sequence (SEQUENCE ID NO: 5) derived therefrom.

E3

Figure 2 shows the contig map depicting the formation of the consensus nucleotide sequence (SEQUENCE ID NO: 5) from the nucleotide alignment of overlapping clones (SEQUENCE ID NO: 1), g2356393 (SEQUENCE ID NO: 2), 1792994 (SEQUENCE ID NO: 3), and 824664IH (SEQUENCE ID NO: 4)."

Please amend page 9, line 32 through page 10, line 2 to read as follows:

"The present invention provides a gene, or a fragment thereof, which codes for a PS128 polypeptide having at least about 50% identity to SEQUENCE ID NO: 12. the present invention further encompasses a PS128 gene, or a fragment thereof, comprising DNA which has at least about 50% identity with SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5."

E4

Please amend page 13, lines 6-21 to read as follows:

""Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide encoded by the sequence. Thus, a "polypeptide,"

E5

"protein," or "amino acid" sequence has at least about 50% identity, preferably about 60% identity, more preferably about 75-85% identity, and most preferably about 90-95% or more identity to a PS128 amino acid sequence. Further, the PS128 "polypeptide," "protein," or "amino acid" sequence may have at least about 60% similarity, preferably at least about 75% similarity, more preferably about 85% similarity, and most preferably about 95% or more similarity to a polypeptide or amino acid sequence of PS128. This amino acid sequence can be selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof."

E5

Please amend page 38, lines 13-30 to read as follows:

"Plasmid pINCY is generally identical to the plasmid pSPORT1 (available from Life Technologies, Gaithersburg, MD) with the exception that it has two modifications in the polylinker (multiple cloning site). These modifications are (1) it lacks a HindIII restriction site and (2) its EcoRI restriction site lies at a different location. pINCY is created from pSPORT1 by cleaving pSPORT1 with both HindIII and EcoRI and replacing the excised fragment of the polylinker with synthetic DNA fragments (SEQUENCE ID NO: 6 and SEQUENCE ID NO: 7). This replacement may be made in any manner known to those of ordinary skill in the art. For example, the two nucleotide sequences, SEQUENCE ID NO: 6 and SEQUENCE ID NO: 7, may be generated synthetically with 5' terminal phosphates, mixed together, and then ligated under standard conditions for performing staggered end ligations into the pSPORT1 plasmid cut with HindIII and EcoRI. Suitable host cells (such as E. coli DH5μ cells) then are transfected with the ligated DNA and recombinant clones are selected for ampicillin resistance. Plasmid DNA then is prepared from individual clones and subjected to restriction enzyme analysis or DNA sequencing in order to confirm the presence of insert sequences in the proper orientation. Other cloning strategies known to the ordinary artisan also may be employed."

E6

Please amend page 42, lines 1-15 to read as follows:

"The cDNA inserts from random isolates of the prostate tissue libraries were sequenced in part, analyzed in detail as set forth in the Examples, and are disclosed in the Sequence Listing as SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3. Also analyzed in detail as set forth in the Examples, and disclosed in the Sequence Listing, is the full-length sequence of the clone 824664 (referred to herein as clone 824664IH (SEQUENCE ID NO: 4)). The consensus sequence of these inserts is presented as SEQUENCE ID NO: 5. These polynucleotides may contain an entire open reading frame with or without associated regulatory sequences for a particular gene, or they may encode only a portion of the gene of interest. This is attributed to the fact that many genes are several hundred and sometimes several thousand bases in length and, with current technology, cannot be cloned in their entirety because of vector limitations, incomplete reverse transcription of the first strand, or incomplete replication of the second strand. Contiguous, secondary clones containing additional nucleotide sequences may be obtained using a variety of methods known to those of skill in the art."

E7

Please amend page 46, lines 10-30 to read as follows:

"For example, antibodies generated against a polypeptide comprising a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal such as a mouse, rabbit, goat or human. A mouse, rabbit or goat is preferred. The polypeptide is selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. The antibody so obtained then will bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies that bind the native polypeptide. Such antibodies then can be used to isolate the polypeptide from test samples such as tissue suspected of containing

E8

that polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique as described by Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique as described by Kozbor et al., Immun. Today 4:72 (1983) and the EBV-hybridoma technique to produce human monoclonal antibodies as described by Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Kiss, Inc., New York, NY, pp. 77-96 (1985). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Patent No. 4,946,778, which is incorporated herein by reference."

E8

Please amend page 49, lines 1-24 to read as follows:

"It is contemplated and within the scope of the present invention that PS128 antigen may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which peptide comprises an amino acid sequence of PS128. The amino acid sequence of such a polypeptide is selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides, identifying different epitopes of PS128, can be used in combination in an assay for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases and conditions of the prostate, such as prostate cancer. In this case, all of these peptides can be coated onto one solid phase; or each separate peptide may be coated onto separate solid phases, such as microparticles, and then combined to form a mixture of peptides which can be later used in assays. Furthermore, it is contemplated that multiple peptides which define epitopes from different antigens may be used for the detection, diagnosis, staging, monitoring, prognosis, prevention or treatment of, or determining the predisposition to,

E9

diseases and conditions of the prostate, such as prostate cancer. Peptides coated on solid phases or labeled with detectable labels are then allowed to compete with those presenting a patient sample (if any) for a limited amount of antibody. A reduction in binding of the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of PS128 antigen in the patient sample. The presence of PS128 antigen indicates the presence of prostate tissue disease, especially prostate cancer, in the patient. Variations of assay formats are known to those of ordinary skill in the art and many are discussed herein below."

E9

Please amend page 50, line 13 through page 51, line 12 to read as follows:

"In yet other assay formats, the polypeptides disclosed herein may be utilized to detect the presence of anibody against PS128 antigen in test samples. For example, a test sample is incubated with a solid phase to which at least one polypeptide such as a recombinant protein or synthetic peptide has been attached. The polypeptide is selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. These are reacted for a time and under conditions sufficient to form antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached, and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the presence of antibody against PS128 antigen. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated. These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the

E10

solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared solid phase, and following standard incubation and washing steps as deemed or required, a recombinant protein derived from a different source (i.e., non-Ecoli) is utilized as a part of an indicator reagent which subsequently is detected. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for PS128 produced or derived from a first source as the capture antigen and an antigen specific for PS128 from a different second source is contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458, which enjoys common ownership and is incorporated herein by reference."

E10

Please amend page 52, line 27 through page 53, line 19 to read as follows:

"It is contemplated that the reagent employed for the assay can be provided in the form of a test kit with one or more containers such as vials or bottle, with each container containing a separate reagent such as a probe, primer, monoclonal antibody or a cocktail of monoclonal antibodies, or a polypeptide (e.g. recombinantly, synthetically produced or purified) employed in the assay. The polypeptide is selected from the group consisting of SEQUENCE ID NO: 12, SEQUENCE ID NO: 13, SEQUENCE ID NO: 14, and fragments thereof. Other components such as buffers, controls and the like, known to those of ordinary skill in art, may be included in such test kits. It also is contemplated to provide test kits which have means for collecting test samples comprising accessible body fluids, e.g., blood, urine, saliva and stool. Such tools

E11

useful for collection ("collection materials") include lancets and absorbent paper or cloth for colleting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Test kits designed for the collection, stabilization and preservation of test specimens obtained by surgery or needle biopsy are also useful. It is contemplated that all kits may be configured in two components which can be provided separately; one component for collection and transport of the specimen and the other component for the analysis of the specimen. The collection component, for example, can be provided to the open market user while the components for analysis can be provided to others such as laboratory personnel for determination of the presence, absence or amount of analyte. Further, its for the collection, stabilization and preservation of test specimens may be configured for use by untrained personnel and may be available in the open market for use at home with subsequent transportation to a laboratory for analysis of the test sample."

E11

Please amend page 54, line 3 through page 55, line 33 to read as follows:

"A. Library Comparison of Expressed Sequence Tags (EST's) or Transcript Images. Partial sequences of cDNA clone inserts, so-called "expressed sequence tags" (EST's), were derived from cDNA libraries made from prostate tumor tissues, prostate non-tumor tissues and numerous other tissues, both tumor and non-tumor and entered into a database (LIFESEQ™ database, available from Incyte Pharmaceuticals, Palo Alto, CA) as gene transcript images. See International Publication No. WO 95/20681. (A transcript image is a listing of the number of EST's for each of the represented genes in a given tissue library. EST's sharing regions of mutual sequence overlap are

E12

classified into clusters. A cluster is assigned a clone number from a representative 5' EST. Often, a cluster of interest can be extended by comparing its consensus sequence with sequences of other EST's which did not meet the criteria for automated clustering. The alignment of all available clusters and single EST's represent a contig from which a consensus sequence is derived.) The transcript images then were evaluated to identify EST sequences that were representative primarily of the prostate tissue libraries. These target clones then were ranked according to their abundance (occurrence) in the target libraries and their absence from background libraries. Higher abundance clones with low background occurrence were given higher study priority. EST's corresponding to the consensus sequence of PS128 were found in 32.3% (11 of 34) of prostate tissue libraries. EST's corresponding to the consensus sequence SEQUENCE ID NO: 5 (or fragments thereof) were found in 0.67% (4 of 599) of the other, non-prostate, libraries of the data base. Therefore, the consensus sequence or fragment thereof was found more than 48 times more often in prostate than non-prostate tissues. Overlapping clones 824664 (SEQUENCE ID NO: 1), g2356393 (SEQUENCE ID NO: 2), and 1792994 (SEQUENCE ID NO: 3) were identified for further study. These represented the minimum number of clones that were needed to form the contig and from which, along with the full-length sequence clone 824664IH (SEQUENCE ID NO: 4), the consensus sequence provided herein (SEQUENCE ID NO: 5) was derived.

E12

B. Generation of a Consensus Sequence. The nucleotide sequences of clones 824664 (SEQUENCE ID NO: 1), g2356393 (SEQUENCE ID NO: 2), 1792994 (SEQUENCE ID NO: 3), and 824664IH (SEQUENCE ID NO: 4) were entered in the Sequencher™ Program (available from Gene Codes Corporation, Ann Arbor, MI) in order to generate a nucleotide alignment (contig map) and then generate their consensus sequence (SEQUENCE ID NO: 5). Figure 1 shows the nucleotide sequence alignment of these clones and their resultant nucleotide consensus sequence (SEQUENCE ID NO: 5). Figure 2 presents the contig map depicting the clones 824664 (SEQUENCE ID NO: 1), g2356393 (SEQUENCE ID NO: 2), 1792994 (SEQUENCE ID NO: 3), and 824664IH (SEQUENCE ID NO:

4), which form overlapping regions of the PS128 gene, and the resultant consensus nucleotide sequence (SEQUENCE ID NO: 5) of these clones in a graphic display. Following this, a three-frame translation was performed on the consensus sequence (SEQUENCE ID NO: 5). The first forward frame was found to have an open reading frame encoding a 57 residue amino acid sequence which is presented as SEQUENCE ID NO: 12.

E12

Analysis of the LIFESEQ™ database indicates a possible C/T polymorphism at position 54 in the consensus nucleotide sequence (SEQUENCE ID NO: 5). There were ten occurrences of each nucleotide in the database. A polymorphism at nucleotide 54 results in an amino acid shift between praline (CCT) and leucine (CTT).

Example 2: Sequencing of PS128 EST-Specific Clones

The DNA sequence of clone 824664IH of the PS128 gene contig was determined (SEQUENCE ID NO: 4) using dideoxy termination sequencing with dye terminators following known methods (F. Sanger et al., PNAS U.S.A. 74:5463 (1977).

Because the pINCY vector (available from Incyte Pharmaceuticals, Inc., Palo Alto, CA) contains universal priming sites just adjacent to the 3' and 5' ligation junctions of the inserts, approximately 300 bases of the insert were sequenced in both directions using two universal primers (SEQUENCE ID NO: 8 and SEQUENCE ID NO: 9, available from New England Biolabs, Beverly, MA, and Applied Biosystems Inc., Foster City, CA, respectively). The sequencing reactions were run on a polyacrlamide denaturing gel, and the sequences were determined by an Applied Biosystems 377 Sequencer (available from Applied Biosystems, Foster City, CA). These primers were sufficient to determine the entire sequence given in SEQUENCE ID NO: 4."

Please amend page 60, lines 26-30 to read as follows:

"Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, is indicative of the presence of PS128 mRNAs, suggesting a diagnosis of a prostate tissue disease or condition, such as prostate cancer."

E13

Please amend page 62, lines 1-5 to read as follows:

"Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, is indicative of the presence of PS128 mRNAs, suggesting a diagnosis of a prostate tissue disease or condition, such as prostate cancer."

E14

Please amend page 62, lines 8-22 to read as follows:

"Dot and slot blot assays are quick methods to evaluate the presence of a specific nucleic acid sequence in a complex mix of nucleic acid. To perform such assays, up to 50 µg of RNA are mixed in 50 µl of 50% formamide, 7% formaldehyde, 1X SSC, incubated 15 min at 68°C, and then cooled on ice. Then, 100 µl of 20X SSC are added to the RNA mixture and loaded under vacuum onto a manifold apparatus that has a prepared nitrocellulose or nylon membrane. The membrane is soaked in water, 20 X SSC for 1 hours, placed on two sheets of 20X SSC prewet Whatman #3 filter paper, and loaded into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as described in Example 4, supra. Detection of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, is indicative of the presence of PS128 mRNAs, suggesting a diagnosis of a prostate tissue disease or condition, such as prostate cancer."

E15

Please amend page 63, lines 25-29 to read as follows:

E16

"Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, is indicative of the presence of PS128 mRNAs, suggesting a diagnosis of a prostate tissue disease or condition, such as prostate cancer."

Please amend page 64, lines 15-29 to read as follows:

E17

"B. Traditional RT-PCR. A traditional two-step RT-PCR reaction was performed, as described by K.W. Hue et al., Virology 181:721-726 (1991). Briefly, 1.0 µg of extracted mRNA (see Example 3) was reverse transcribed in a 20 µl reaction mixture containing 1X PCR II buffer (Perkin-Elmer), 5 mM $MgCL_2$, 1 mM dNTP, 20 U RNasin, 2.5 µM random hexamers, and 50 U MMLV (Moloney murine leukemia virus) reverse transcriptase (RT). Reverse transcription was performed at room temperature for 10 min, 42°C for 30 min in a PE-480 thermal cycler, followed by further incubation at 95°C for 5 min to inactivate the RT. PCR was performed using 2 µl of the cDNA reaction in a final PCR reaction volume of 50 µl containing 10 mM Tris0HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCL_2$, 200 $MgCL_2$, 1M dNTP, 0.4 $MgCL_2$, 1M of each sense and antisense primer, SEQUENCE ID NO: 10 and SEQUENCE ID NO: 11, respectively, and 2.5 U of Taq polymerase. The reaction was incubated in an MJ Research Model PTC-200 as follows: Denaturation at 94°C, 45 sec; 55°C, 45 sec; 72°C, 2 min); a final extension (72°C, 5 min); and a soak at 4°C."

Please amend page 65, lines 18-22 to read as follows:

E18

"Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO:

E18 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, is indicative of the presence of PS128 mRNAs, suggesting a diagnosis of a prostate tissue disease or condition, such as prostate cancer."

Please amend page 66, lines 34-35 to read as follows:

E19 "Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, "

Please amend page 67, lines 1-3 to read as follows:

E20 "SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, is indicative of the presence of PS128 mRNAs, suggesting a diagnosis of a prostate tissue disease or condition, such as prostate cancer."

Please amend page 67, lines 6-19 to read as follows:

"Synthetic peptides were modeled and then prepared based upon the predicted amino acid sequence of the PS128 polypeptide consensus sequence (see Example 1). In particular, a number of PS128 peptides derived from SEQUENCE ID NO: 12 were prepared, including the peptides of SEQUENCE ID NO: 13 and SEQUENCE ID NO: 14. All peptides were sunthesized on a

E21 Symphony Peptide Synthesizer (available from Rainin Instrument Co, Emeryville, CA) using FMOC chemistry, standard cycles and in-situ HBTU activation. Cleavage and deprotection conditions were as follows: a volume of 2.5 ml of cleavage reagent (77.5% v/v trifluoroacetic acid, 15% v/v ethanedithiol, 2.5% v/v water, 5% v/v thioanisole, 1-2% w/v phenol) were added to the resin, and agitated at room temperature for 2-4 hours. Then the filtrate was removed and the peptide was precipitated from the cleavage reagent with cold diethyl ether.

Each peptide was filtered, purified via reverse-phase preparative HPLC using a water/acetonitrile/0.1% TFA gradient, and then lyophilized. The product was confirmed by mass spectrometry."

Please amend page 68, lines 5-32 to read as follows:

"Plasmids for the expression of secretable PS128 proteins are constructed by replacing the hepatitis C virus E2 protein coding sequence in plasmid 577 with that of a PS128 polynucleotide sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof, as follows. Digestion of plasmid 577 with XbaI releases the hepatitis C virus E2 gene fragment. The resulting plasmid backbone allows insertion of the PS128 cDNA insert downstream of the rabbit immunoglobulin heavy chain signal sequence which directs the expressed proteins into the secretory pathway of the cell. The PS128 cDNA fragment is generated by PCR using standard procedures. Encoded in the sense PCR primer sequence is an XbaI site, immediately followed by a 12 nucleotide sequence that encodes the amino acid sequence Ser-Asn-Glu-Leu ("SNEL") to promote signal protease processing, efficient secretion and final product stability in culture fluids. Immediately following this 12 nucleotide sequence the primer contains nucleotides complementary to template sequences encoding amino acids of the PSS128 gene. The antisense primer incorporates a sequence encoding the following eight amino acids just before the stop codons: Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQUENCE ID NO: 15). Within this sequence is incorporated a recognition site to aid in analysis and purification of the PS128 protein product. A recognition site (termed "FLAG") that is recognized by a commercially available monoclonal antibody designated anti-FLAG M2 (Eastman Kodak, Co., New Haven, CT) can be utilized, as well as other comparable sequences and their corresponding antibodies. For example, PCR is performed using GeneAmp® reagents obtained from Perkin-Elmer-Cetus, as directed by the supplier's

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instructions. PCR primers are used at a final concentration of 0.5 µM. PCR is performed on the PS128 plasmid template in a 100 µl reaction for 35 cycles (94°C, 30 seconds; 55°C, 30 seconds; 72°C, 90 seconds) followed by an extension cycle of 72°C for 10 min."

E22

Please amend page 71, lines 16-21 to read as follows:

"Plasmids for the expression of secretable PS128 proteins are constructed by inserting a PS128 polynucleotide sequence selected from the group consisting of SEQUENCE ID NO: 1, SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4, SEQUENCE ID NO: 5, and fragments or complements thereof. Prior to construction of a PS128 expression plasmid, the PS128 cDNA sequence is first cloned into a pCR®-Blunt vector as follows:"

E23

Please amend page 77, lines 27-33 to read as follows:

"A. Production of Polyclonal Antisera. Antiserum against PS128 was prepared by injecting rabbits with peptides whose sequences were derived from that of the predicted amino acid sequence of the PS128 consensus nucleotide sequence (SEQUENCE ID NO: 5). The synthesis of peptides (SEQUENCE ID NO: 13, and SEQUENCE ID NO: 14) is described in Example 10. Peptides used as immunogens were not conjugated to a carrier such as keyhole limpet hemocyanine, KLH, (i.e., they were unconjugated.)."

E24

Please amend page 77, line 34 through page 78, line 22 to read as follows:

"For animal immunizations, female white New Zealand rabbits weighing 2 kg or more were used for raising polyclonal antiserum. One animal was immunized per unconjugated peptide (SEQUENCE ID NO: 13, or SEQUENCE ID NO: 14). One week prior to the first immunization, a 5 to 10 ml blood sample was obtained from each animal to serve as a non-immune prebleed sample.

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Unconjugated PS128 peptides of SEQUENCE ID NO: 13 and SEQUENCE ID NO: 14 were used to prepare the primary immunogen by emulsifying 0.5 ml of the peptide at a concentration of 2 mg/ml in PBS (pH 7.2) which contained 0.5 ml of complete Freund's adjuvant (CFA) (Difco, Detroit, MI). The immunogen was injected into several sites of the animal via subcutaneous, intraperitoneal, and intramuscular routes of administration. Four weeks following the primary immunization, a booster immunization was administered. The immunogen used for the booster immunization dose was prepared by emulsifying 0.5 ml of the samel unconjugated peptides used for the primary immunogen, except that the peptide now was diluted to 1 mg/ml with 0.5 ml of incomplete Freund's adjuvant (IFA) (Difco, Detroit, MI). Again, the booster dose was administered into several sites via subcutaneous, intraperitoneal and intramuscular types of injections. The animals were bled (5 ml) two weeks after the booster immunizations and each serum was tested for immunoreactivity to the peptide as described below. The booster and blled schedules were repeated at 4 week intervals until an adequate titer was obtained. The titer or concentration of antiserum was determined by using unconjugated peptides in a microtiter EIA as described in Example 17, below. An antibody titer of 1:500 or greater was considered an adequate titer for further use and study."

E25

Please amend page 78, lines 25 – 30 to read as follows:

E26

"**Peptide Immunogen**	**Titer**
SEQUENCE ID NO: 13	>62,500
SEQUENCE ID NO: 14	10,400"

Please amend page 84, lines 2-33 to read as follows:

E27

"The immunoreactivity of antiserum obtained from rabbits as described in Example 14 was determined by means of a microtiter plate EIA, as follows. Briefly, synthetic PS128 peptides (SEQUENCE ID NO: 13 and SEQUENCE ID

NO: 14) prepared as described in Example 10, were dissolved in carbonate buffer (50 mM, pH 9.6) to a final concentration of 2 µg/ml. Next, 100 µl of the peptide or protein solution were placed in each well of an Immulon 2® microtiter plate (Dynex Technologies, Chantilly, VA). The plate was incubated overnight at room temperature and then washed four times with deionized water. The wells were blocked by adding 125 µl of a suitable protein blocking agent, such as Superblock® (Pierce Chemical Company, Rockford, IL), to each well and then immediately discarding the solution. This blocking procedure was performed three times. Antiserum obtained from immunized rabbits or mice, prepared as previously described, was diluted in a protein blocking agent (e.g., a 3% Superblock® solution) in PBS containing 0.05% Tween-20® (monolaurate polyoxyethylene ether) (Sigma Chemical Company, St. Louis, MO) and 0.05% sodium azide at dilutions of 1:100, 1:500, 1:2500, 1:12,500, and 1:62,500 and placed in each well of the coated microtiter plate. The wells then were incubated for three hours at room temperature. Each well was washed four times with deionized water. One hundred microliters of alkaline phosphatase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG antiserum (Southern Biotech, Birmingham, AB) diuted 1:2000 in 3% Superblock® solution in phosphate buffered saline containing 0.05% Tween 20® and 0.05% sodium azide, were added to each well. The wells were incubated for two hours at room temperature. Next, each well was washed four times with deionized water. One hundred microliters of paranitrophenyl phosphate substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) then were added to each well. The wells were incubated for thirty minutes at room temperature. The absorbance at 405 nm was read in each well. Positive reactions were identified by an increase in absorbance at 405 nm in the test well above that absorance given by a non-immune serum (negative control). A positive reaction was indicative of the presence of detectable anti-PS128 antibodies. Titers of the anti-peptide antisera were calculated from the previously described dilutions of antisera and defined as the calculated dilution, where A_{405nm}=0.5 OD."

E27